☐ 1: Lab Chip. 2001 Dec;1(2):153-7. Epub 2001 Nov 26.

Enzyme linked immunosorbent assay on a microchip with electrochemica detection.

Rossier JS, Girault HH.

Departement de Chimie, Ecole Polytechnique Federale de Lausanne, CH-1015, Lausann Switzerland.

This paper presents the development of a sandwich immunoassay in disposable plastic microchips. Photoablated microchannels with integrated electrodes have been used for th development of enzyme-linked-immunosorbent-assay (ELISA). The presence of the electrode inside the 40 nL microchannel enables the detection of the redox active enzym substrate directly inside the reaction channel. Furthermore, due to the small diffusion distances, each incubation time can be reduced to five minutes instead of a few hours in standard microtiterplates. The initial characterisation of this immunoassay has been performed with a large protein complex D-Dimer-alkaline phosphatase. This system was used for the detection of immobilised antibodies on the surface of the photoablated microchannel. In a second step, a sandwich immunoassay with a horseradish peroxidase secondary antibody conjugate (HRP-conjugate) was used to detect D-Dimer between 0.1 and 100 nM, which is the relevant concentration range of the clinical tests.

PMID: 15100877 [PubMed - indexed for MEDLINE]

h cb h g e e e fcg c e e e b b e b c

☐ 1: J Immunoassay. 1992;13(2):231-52. Related Articles, L

A rapid and sensitive heterogeneous immunoelectrochemical assay using disposable electrodes.

Hadas E, Soussan L, Rosen-Margalit I, Farkash A, Rishpon J.

Department of Biotechnology, Faculty of Life Sciences, Tel-Aviv University, Israel.

In this novel enzyme-tagged immuoelectrochemical assay, disposable carbon felt discs serve both as electrodes and as the heterogeneous solid phase. Antibodies are immobilize on the carbon felt via a diaminoalkane-biotin-avidin-biotin bridge. Alkaline phosphatase used as a label. Bound antibodies are monitored by following the electro-oxidation of aminophenol, produced enzymatically from p-amino-phenyl phosphate by the immobiliz alkaline phosphatase at the electrode surface. A model system designed for determinatio of mouse IgG concentration yielded a calibration curve ranging from 10 pg/ml to 100 micrograms/ml. This assay can be performed rapidly and a single determination complet within 20 minutes. The system is useful also for rapid quantitation of a small number (approximately 80 organisms per ml) of bacteria.

PMID: 1430241 [PubMed - indexed for MEDLINE]

h cb hg e e e fcg c e e e b b e b c

☐ 1: Anal Chem. 2001 Nov 1;73(21):5323-7.

Electrochemical enzyme immunoassays on microchip platforms.

Wang J, Ibanez A, Chatrathi MP, Escarpa A.

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces 88003, USA.

A microfluidic device for conducting electrochemical enzyme immunoassays is describe The new "lab-on-a-chip" protocol integrates precolumn reactions of alkaline phosphatase labeled antibody (anti-mouse IgG) with the antigen (mouse IgG), followed by electrophoretic separation of the free antibody and antibody-antigen complex. The separation is followed by a postcolumn reaction of the enzyme tracer with the 4-aminophenyl phosphate substrate and a downstream amperometric detection of the liberated 4-aminophenol product Factors influencing the reaction, separation, and detecti processes were optimized, and the analytical performance was characterized. An applied field strength of 256 V/cm results in free antibody and antibody-antigen complex migrat times of 125 and 340 s, respectively. A remarkably low detection limit of 2.5 x 10(-16) g/mL (1.7 x 10(-18) M) is obtained for the mouse IgG model analyte. Such combination a complete integrated immunoassay, an attractive analytical performance, and the distinc miniaturization/portability advantages of electrochemical microsystems offers considera promise for designing self-contained and disposable chips for decentralized clinical diagnostics or on-site environmental testing.

PMID: 11721936 [PubMed - indexed for MEDLINE]

☐ 1: Scand J Clin Lab Invest. 1986 Dec;46(8):751-9. Related Articles, L

Monoclonal antibodies based sandwich erythro-immunoassay and 'dot' enzyme immunoassay for human chorionic gonadotropin in urine.

Gupta SK, Talwar GP.

Two simple solid-phase sandwich immunoassays for human chorionic gonadotropin (hC employing monoclonal antibodies have been described. One is a sandwich erythro-immunoassay employing V-shaped well microtitration plates coated with monoclonal an beta-hCG antibody and monoclonal anti-alpha-hCG antibody labelled sheep erythrocyte The second is a 'dot' enzyme immuno-assay employing dip-stick (plastic strips pasted wi nitrocellulose pads) coated with monoclonal anti-beta-hCG antibody. Anti-alpha-hCG monoclonal-alkaline phosphatase conjugate was used to reveal hCG bound to solid surfa The assays can be performed by 'one-step' or 'two-step' procedures. Erythro-immunoassa as well as 'dot' enzyme immunoassay was able to detect in urine as low as 10 mIU hCG/ A good correlation was observed between the values obtained by these two methods as well as 'two-step' sandwich enzyme immunoassay on 47 urine samples.

PMID: 3797987 [PubMed - indexed for MEDLINE]

h cb h g e e e fcg c e e e b b e b c

1: Clin Chem. 1995 Sep;41(9):1378-84.

Novel nonseparation sandwich-type electrochemical enzyme immunoassay system for detecting marker proteins in undiluted blood.

Meyerhoff ME, Duan C, Meusel M.

Department of Chemistry, University of Michigan, Ann Arbor 48109, USA.

A novel nonseparation electrochemical enzyme immunoassay (NEEIA) for detecting marker proteins in undiluted blood is described. The approach is based on preferential electrochemical measurement of surface-bound enzyme-labeled reporter antibody (E-Ab relative to an excess of this reagent in the sample solution. NEEIAs are carried out on microporous membranes coated with a thin, circular area of gold. The gold serves simultaneously as a working electrode and solid phase for immobilized capture anti-prot antibodies. In the assay, analyte protein is incubated concurrently with the Ab-coated go surface and excess E-Ab conjugate. Detection of bound E-Ab is achieved by introducing the substrate for the enzyme through the back side of the membrane. The product of bou E-Ab is detected immediately by oxidation or reduction at the gold electrode, and the resulting current is proportional to the concentration of protein in the sample. The feasibility of the NEEIA approach is demonstrated via the detection of prostate-specific antigen in undiluted plasma samples (n = 64), with alkaline phosphatase as the label. Use of multiple gold films deposited on the same porous membrane to perform simultaneous NEEIAs is also described.

PMID: 7544708 [PubMed - indexed for MEDLINE]

1: Anal Chem. 1994 May 1;66(9):1369-77. Related Articles, L

Separation-free sandwich enzyme immunoassays using microporous gold electrodes and self-assembled monolayer/immobilized capture antibodies.

Duan C, Meyerhoff ME.

Department of Chemistry, University of Michigan, Ann Arbor 48109.

A novel separation-free sandwich-type enzyme immunoassay for proteins is performed b designing an electrochemical detection system that enables preferential measurement of surface-bound enzyme-labeled antibody relative to the excess enzyme-labeled reagent in the bulk sample solution. In this initial model system, the assay is carried out using gold coated microporous nylon membranes (pore size 0.2 micron) which are mounted betwee two chambers of a diffusion cell. The membrane serves as both a solid phase for the sandwich assay and the working electrode in the three-electrode amperometric detection system. The capture monoclonal antibody is immobilized covalently on the gold side of membrane via a self-assembled monolayer of thioctic acid. In the separation-free sandwi assay, both model analyte protein (human chorionic gonadotropin; hCG) and alkaline phosphatase labeled anti-hCG (ALP-Ab) are incubated simultaneously with the immobilized capture anti-hCG antibody. Surface-bound ALP-Ab is spatially resolved fro the excess conjugate in the bulk sample solution by introducing the enzyme substrate (4-aminophenyl phosphate) through the back side of the porous membrane. The substrate diffuses rapidly through the porous membrane where it first encounters bound ALP-Ab a the gold surface. The enzymatically generated product, aminophenol, is detected immediately by oxidation at the gold electrode (at +0.19 V vs Ag/AgCl), and the magnitude of current is directly proportional to the concentration of hCG in the sample. The response time after substrate addition is less than 1 min, although maximum respons toward the analyte protein requires a sample/conjugate preincubation time of 30 min wit the porous electrode.(ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 8017631 [PubMed - indexed for MEDLINE]

☐ 1: Biosens Bioelectron. 2000 Jun;15(3-4):183-92. Related Articles, L

Nature of immobilized antibody layers linked to thioctic acid treated gold surfaces.

Smith AM, Ducey MW Jr, Meyerhoff ME.

Department of Chemistry, The University of Michigan, Ann Arbor 48109-1055, USA.

Utilization of 125I-labeled IgG enables an investigation of protein immobilized to gold electrodes sputter deposited on microporous nylon membranes, including the precise nat of the surface-protein bond (i.e. covalent or non-specific adsorption), physical location o the immobilized protein (i.e. on the surface of the gold electrode or within the pores of th membrane), and the amount of protein immobilized. This is accomplished by comparing the mass of protein immobilized to gold surfaces that have been treated in several differe fashions, as well as, deposition of the gold on nylon membranes that have been treated differently. It is shown that these microporous gold electrodes, proposed previously for conducting novel non-separation electrochemical enzyme immunoassays, consist of multiple protein layers non-specifically adsorbed. Approximately, half of the total adsorb protein is immobilized to the gold surface with the remaining protein bound within the pores on the nylon membrane.

PMID: 11286336 [PubMed - indexed for MEDLINE]

h cb hg e e e fcg c e e e b b e b c

☐ 1: Biosensors. 1986;2(1):45-57. Related Articles, L

Bioelectrochemical immunoassay for human chorionic gonadotrophin in serum using an electrode-immobilised capture antibody.

Robinson GA, Cole VM, Rattle SJ, Forrest GC.

An amperometric technique for the quantification of an enzyme immunoassay which utilises a capture antibody covalently attached to a carbon electrode is described. The electrode is used both to separate the assay and to monitor the activity of the bound enzy label. A 'two-site' immunometric assay with monoclonal antibodies directed against hum chorionic gonadotrophin (HCG) was used as the model system. The activity of the enzym bound to the electrode is determined electrochemically by the use of an electron transfer mediator (dimethylaminomethyl ferrocene) permitting rapid quantification of the analyte without the need for a separate incubation step to measure enzyme activity. The sensitivi of the assay is 9mIU HCG ml-1 in serum (1st International Reference Preparation). The correlation between the amperometric measurement of serum HCG and data for an immunoradiometric assay was r = 0.988. The assay is rapid requiring a total assay time o 20 min per sample, which includes 15 min for antibody-antigen binding.

PMID: 3790178 [PubMed - indexed for MEDLINE]

☐ **1:** Anal Biochem. 1991 May 1;194(2):295-301.

Enzyme-amplified rate conductimetric immunoassay.

Thompson JC, Mazoh JA, Hochberg A, Tseng SY, Seago JL.

E. I. Du Pont de Nemours and Company, Inc., Medical Products, Newark, Delaware.

A new immunoassay technique based on measurement of conductance changes in solutio is described. The assay employs an immobilized monoclonal antibody to capture a prote analyte along with a second antibody to the same analyte, conjugated to an enzyme capa of producing ions which are measured conductimetrically. Urease was selected as the enzyme, because it produces, from urea, four ions for each catalytic event. The analyte studied was human chorionic gonadotropin in serum. Higher concentrations of analyte during incubation with immobilized antibody and antibody-urease conjugate led to increased binding of the latter. After removal of unbound conjugate, urea solution was added and the rate of conductance change measured in the bulk substrate solution. Experiments, performed in polystyrene microtiter wells using a specially designed electrode, demonstrated the ability to measure 30 picomolar concentrations of human chorionic gonadotropin with a 30-s rate measurement. Urease proved to be an excellent labeling enzyme, retaining its activity under the nonionic conditions necessary to mainta low background conductance. Good agreement was obtained between observed rates and those expected from conductimetric theory and known physical parameters. The potentia utility of the conductimetric immunoassay lies in the fabrication of biosensor devices for simplification and cost reduction of immunochemical-based instrumentation. Further improvements to the technique are proposed to achieve lower detection limits.

PMID: 1862933 [PubMed - indexed for MEDLINE]

h cb h g e e e fcg c e e e b b e b c

☐ 1: Toxicon. 2002 Mar;40(3):255-8. Related Articles, L

Colloidal gold-based immunochromatographic assay for detection of ricin

Shyu RH, Shyu HF, Liu HW, Tang SS.

Institute of Preventive Medicine, National Defense Medical Center, Taipei, Taiwan, Republic of China.

A rapid immunochromatographic assay was developed to detect ricin. The assay was bas on the sandwich format using monoclonal antibodies (Mabs) of two distinct specificities One anti-ricin B chain Mab (1G7) was immobilized to a defined detection zone on a por nitrocellulose membrane, while the other anti-ricin A chain Mab (5E11) was conjugated colloidal gold particles which served as a detection reagent. The ricin-containing sample was added to the membrane and allowed to react with Mab (5E11)-coated particles. The mixture was then passed along the porous membrane by capillary action past the Mab (1G7) in the detection zone, which will bind the particles that had ricin bound to their surface, giving a red color within this detection zone with an intensity proportional to ric concentration. In the absence of ricin, no immunogold was bound to the solid-phase antibody. With this method, 50 ng/ml of ricin was detected in less than 10 min. The assa sensitivity can be increased by silver enhancement to 100 pg/ml.

PMID: 11711121 [PubMed - indexed for MEDLINE]
